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(71) Applicant: ABBOTT LABORATORIES [US/US]; CHAD 0377/AP6D–2, 100 Abbott Park Road, Abbott Park, IL 60064–6050 (US).

(72) Inventors: LYNCH, Kevin, J.; 17904 West Hampshire Drive, Gurnee, IL 60031 (US). BURGARD, Edward, C.; 609 Burdick Street, Libertyville, IL 60048 (US). METZGER, Randy, E.; 225 Saratoga Court, Gurnee, IL 60031 (US). NIFORATOS, Wende; 2208 W. Wilson, Chicago, IL 60625 (US). TOUMA, Edward, B.; 6116 N. Winchester, Chicago, IL 60660 (US).

(74) Agents: GOLLER, Mimi, C. et al.; Abbott Laboratories, Chad 0377/AP6D–2, 100 Abbott Park Road, Abbott Park, IL 60064–3500 (US).

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(57) Abstract

A human $P2X_2$ purinergic receptor polypeptide is provided. Nucleic acid molecules encoding the human $P2X_2$ receptor polypeptide, and vectors and host cells containing such nucleic acid molecules, are also provided. In addition, methods are provided for producing the $P2X_2$ receptor polypeptide, as are methods of using such polypeptides and host cells that express the same to screen for compounds having activity on $P2X_2$ receptor. Further, therapeutic uses involving aspects of this receptor are contemplated.

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# NUCLEIC ACIDS ENCODING A FUNCTIONAL HUMAN PURINORECEPTOR $P2X_2$ AND METHODS OF PRODUCTION AND USE THEREOF

## Technical Field

The invention relates generally to receptor proteins and to DNA and RNA molecules encoding therefor. In particular, the invention relates to a nucleic acid sequence that encodes a human receptor $P2X_2$. The invention also relates to methods of using the receptor encoded thereby to identify compounds that interact with it. This invention further relates to compounds which act as antagonists and agonists to compounds which have reactivity with the $P2X_2$ receptor and methods utilized in determining said reactivity. The invention also involves therapeutic uses involving aspects of this receptor.

## Background of the Invention

P2 receptors have been generally categorized as either metabotropic nucleotide receptors or ionotropic receptors for extracellular nucleotides. Metabotropic nucleotide receptors (usually designated P2Y or $P2Y_n$, where "n" is a subscript integer indicating subtype) are believed to differ from ionotropic receptors (usually designated P2X or $P2X_n$) in that they are based on a different fundamental means of transmembrane signal transduction: P2Y receptors operate through a G protein-coupled system, while P2X receptors are ligand-gated ion channels. The ligand for these P2X receptors is ATP, and/or other natural nucleotides, for example, ADP, UTP, UDP, or synthetic nucleotides, for example 2-methylthioATP.

At least seven P2X receptors, and the cDNA sequences encoding them, have been identified to date. $P2X_1$ cDNA was cloned from the smooth muscle of the rat vas deferens (Valera *et al.* (1994) Nature 371:516-519) and $P2X_2$ cDNA was cloned from PC12 cells (Brake *et al.* (1994) Nature 371:519-523). Five other P2X receptors have been found in cDNA libraries by virtue of their sequence similarity to $P2X_1$ and $P2X_2$ ($P2X_3$: Lewis *et al.* (1995) Nature 377:432-435, Chen *et al.* (1995) Nature 377:428-431; $P2X_4$: Buell *et al.* (1996) EMBO J. 15:55-62, Seguela *et al.* (1996) J. Neurosci. 16:448-455, Bo *et al.* (1995) FEBS Lett. 375:129-133, Soto *et al.* (1996) Proc. Natl. Acad. Sci. USA 93:3684-3688, Wang *et al.* (1996) Biochem. Biophys. Res. Commun.220:196-202; $P2X_5$: Collo *et al.* (1996) J. Neurosci. 16:2495-2507, Garcia-Guzman *et al.* (1996) FEBS Lett. 388:123-127; $P2X_6$: Collo *et al.* (1996), supra, Soto *et al.* (1996) Biochem. Biophys. Res. Commun. 223:456-460; $P2X_7$: Surprenant *et al.*

(1996) Science 272:735-738). For a comparison of the amino acid sequences of rat P2X receptors see Buell *et al.* (1996) Eur. J. Neurosci. 8:2221-2228.

Native P2X receptors form rapidly activated, nonselective cationic channels that are activated by ATP. Rat $P2X_1$ and rat $P2X_2$ have equal permeability to $Na^+$ and $K^+$ but significantly less to $Cs^+$. The channels formed by the P2X receptors generally have high $Ca^{2+}$ permeability ($P_{Ca}/P_{Na} \cong 4$). The cloned rat $P2X_1$, $P2X_2$ and $P2X_4$ receptors exhibit the same permeability for $Ca^{2+}$ observed with native receptors. However, the mechanism by which P2X receptors form an ionic pore or bind ATP is not known.

A variety of tissues and cell types, including epithelial, immune, muscle and neuronal, express at least one form of P2X receptor. The widespread distribution of $P2X_4$ receptors in the rat central nervous system suggests a role for $P2X_4$-mediated events in the central nervous system. However, study of the role of individual P2X receptors is hampered by the lack of receptor subtype-specific agonists and antagonists. For example, one agonist useful for studying ATP-gated channels is α,β-methylene-ATP (α,βmeATP). However, the P2X receptors display differential sensitivity to the agonist with $P2X_1$ and $P2X_2$ being α,βmeATP-sensitive and insensitive, respectively. Furthermore, binding of α,βmeATP to P2X receptors does not always result in channel opening. The predominant forms of P2X receptors in the rat brain, $P2X_4$ and $P2X_6$ receptors, cannot be blocked by suramin or PPADS. These two forms of the P2X receptor are also not activated by α,βmeATP and are, thus, intractable to study with currently available pharmacological tools.

A therapeutic role for P2 receptors has been suggested, for example, for cystic fibrosis (Boucher *et al.* (1995) in: Belardinelli *et al.* (eds) Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology (Kluwer Acad., Norwell MA) pp 525-532), diabetes (Loubatiéres-Mariani *et al.* (1995) in: Belardinelli *et al.* (eds), supra, pp 337-345), immune and inflammatory diseases (Di Virgilio *et al.* (1995) in: Belardinelli *et al.* (eds), supra, pp 329-335), cancer (Rapaport (1993) Drug Dev. Res. 28:428-431), constipation and diarrhea (Milner *et al.* (1994) in: Kamm *et al.* (eds.) Constipation and Related Disorders: Pathophysiology and Management in Adults and Children (Wrightson Biomedical, Bristol) pp 41-49), behavioral disorders such as epilepsy, depression and aging-associated degenerative diseases (Williams (1993) Drug. Dev. Res. 28:438-444), contraception and sterility (Foresta *et al.* (1992) J. Biol. Chem. 257:19443-19447), and wound healing (Wang *et al.* (1990) Biochim. Biophys. Res. Commun. 166:251-258).

Accordingly, there is a need in the art for specific agonists and antagonists for each $P2X_2$ receptor subtype and, in particular, agents that will be effective *in vivo*, as well as for methods for identifying $P2X_2$ receptor-specific agonist and antagonist compounds.

Summary of the Invention

The present invention relates to a human $P2X_2$ receptor.

In one embodiment, a DNA molecule or fragments thereof is provided, wherein the DNA molecule encodes a human $P2X_2$ receptor or subunit thereof.

In another embodiment, a recombinant vector comprising such a DNA molecule, or fragments thereof, is provided.

In another embodiment, the subject invention is directed to a human $P2X_2$ receptor polypeptide, either alone or in multimeric form.

In still other embodiments, the invention is directed to messenger RNA encoded by the DNA, recombinant host cells transformed or transfected with vectors comprising the DNA or fragments thereof, and methods of producing recombinant $P2X_2$ polypeptides using such cells.

In yet another embodiment, the invention is directed to a method of expressing a human $P2X_2$ receptor, or a subunit thereof, in a cell to produce the resultant $P2X_2$-containing receptor.

In a further embodiment, the invention is directed to a method of using such cells to identify potentially therapeutic compounds that modulate or otherwise interact with the above $P2X_2$-containing receptors.

In another embodiment, therapeutic uses involving a $P2X_2$ modulator, such as an ATP agonist or antagonist are contemplated.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Drawings

FIGURE 1 depicts the partial sequence of a cDNA clone (SEQ ID NO:1) derived from human fetal colon tissue which encodes a polypeptide with homology to a region of the rat $P2X_2$ receptor;

FIGURE 2 depicts the full sequence of the cDNA clone (SEQ ID NO:2), the underlined sequences sequence denotes overlap with the sequence of Figure 1;

FIGURE 3 a-e depicts primers designed to the cDNA of Figure 2 and commercial RACE primers: 3a depicts GSP 1 (SEQ ID NO:3); 3b depicts GSP 2

(SEQ ID NO:4); 3c depicts GSP 3 (SEQ ID NO:5); 3d depicts the anchor primer (SEQ ID NO:6); and 3e depicts the universal amplification primer (SEQ ID NO:7);

FIGURE 4 depicts the approximately 600 bp product (SEQ ID NO:8) produced by 5' RACE reactions using poly A RNA from human pituitary tissue;

FIGURE 5 depicts genomic primers (SEQ ID NO:9 and SEQ ID NO:10);

FIGURE 6 depicts $hP2X_2$ RT-PCR primers (SEQ ID NO:11 and SEQ ID NO:12);

FIGURE 7 a-d depicts four species of cDNAs (SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; and SEQ ID NO:16, respectively) containing intact open reading frames from the predicted initiation to termination sites;

FIGURE 8 a-d depicts the predicted amino acid sequences (SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; and SEQ ID NO:20) encoded by the nucleotides of Figure 7;

FIGURE 9 depicts an alignment of the predicted amino acid sequences (SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; and SEQ ID NO:20); and

FIGURE 10 depicts electrophysiological characterization of $hP2X_2$ channels.

Detailed Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, electrophysiology, and pharmacology, that are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989); DNA Cloning, Vols. I and II (D.N. Glover Ed. 1985); Perbal, B., A Practical Guide to Molecular Cloning (1984); the series, Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Transcription and Translation (Hames *et al.* eds. 1984); Gene Transfer Vectors For Mammalian Cells (J. H. Miller *et al.* eds. (1987) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); Scopes, Protein Purification: Principles and Practice (2nd ed., Springer-Verlag); and PCR: A Practical Approach (McPherson *et al.* eds. (1991) IRL Press).

All patents, patent applications and publications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety and are deemed representative of the prevailing state of the art.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

Thus, for example, reference to "a primer" includes two or more such primers, reference to "an amino acid" includes more than one such amino acid, and the like.

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

The term "P2 receptor" intends a purinergic receptor for the ligand ATP and/or other purine or pyrimidine nucleotides, whether natural or synthetic. P2 receptors are broadly subclassified as "P2X" or "P2Y" receptors. These types differ in their pharmacology, structure, and signal transduction mechanisms. The P2X receptors are generally ligand-gated ion channels, while the P2Y receptors operate generally through a G protein-coupled system. Moreover, and without intending to be limited by theory, it is believed that P2X receptors comprise multimers of receptor polypeptides, which multimers may be of either the same or different subtypes. Consequently, the term "P2X receptor" refers, as appropriate, to the individual receptor subunit or subunits, as well as to the homomeric and heteromeric receptors comprised thereby.

The term "$P2X_n$" intends a P2X receptor subtype wherein n is an integer of at least 1. At the time of the invention, at least 7 $P2X_n$ receptor subtypes have been isolated and/or characterized.

A "$P2X_2$ receptor agonist" is a compound that binds to and activates a $P2X_2$ receptor. By "activates" is intended the elicitation of one or more pharmacological, physiological, or electrophysiological responses. Such responses may include, but are not limited to, an increase in receptor-specific cellular depolarization.

A "$P2X_2$ receptor antagonist" is a substance that binds to a $P2X_2$ receptor and prevents agonists from activating the receptor. Pure antagonists do not activate the receptor, but some substances may have mixed agonist and antagonist properties.

The term "polynucleotide" as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modifications, such as by methylation and/or by capping, and unmodified forms of the polynucleotide.

The term "variant" is used to refer to an oligonucleotide sequence which differs from the related wild-type sequence in the insertion, deletion or substitution of one or more nucleotides. When not caused by a structurally conservative mutation (see below), such a variant oligonucleotide is expressed as a "protein variant" which, as used herein, indicates a polypeptide sequence that differs from the wild-type polypeptide in the insertion, deletion or substitution of one or more amino acids. The protein variant differs in primary structure (amino acid sequence), but may or may not

differ significantly in secondary or tertiary structure or in function relative to the wild-type.

The term "mutant" generally refers to an organism or a cell displaying a new genetic character or phenotype as the result of change in its gene or chromosome. In some instances, however, "mutant" may be used in reference to a variant protein or oligonucleotide and "mutation" may refer to the change underlying the variant.

"Polypeptide" and "protein" are used interchangeably herein and indicate a molecular chain of amino acids linked through peptide bonds. The terms do not refer to a specific length of the product. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide, provided that such fragments, etc. retain the binding or other characteristics necessary for their intended use.

A "functionally conservative mutation" as used herein intends a change in a polynucleotide encoding a derivative polypeptide in which the activity is not substantially altered compared to that of the polypeptide from which the derivative is made. Such derivatives may have, for example, amino acid insertions, deletions, or substitutions in the relevant molecule that do not substantially affect its properties. For example, the derivative can include conservative amino acid substitutions, such as substitutions which preserve the general charge, hydrophobicity/hydrophilicity, side chain moiety, and/or steric bulk of the amino acid substituted, for example, Gly/Ala, Val/Ile/Leu, Asp/Glu, Lys/Arg, Asn/Gln, Thr/Ser, and Phe/Trp/Tyr.

By the term "structurally conservative mutant" is intended a polynucleotide containing changes in the nucleic acid sequence but encoding a polypeptide having the same amino acid sequence as the polypeptide encoded by the polynucleotide from which the degenerate variant is derived. This can occur because a specific amino acid may be encoded by more than one "codon," or sequence of three nucleotides, *i.e.*, because of the degeneracy of the genetic code.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, immaterial of the method by which the DNA is introduced into the cell or the subsequent disposition of the cell. The terms include the progeny of the original cell which has been transfected. Cells in primary culture as well as cells such as oocytes also can be used as recipients.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment. The term includes expression vectors, cloning vectors, and the like.

A "coding sequence" is a polynucleotide sequence that is transcribed into mRNA and/or translated into a polypeptide. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, and recombinant polynucleotide sequences. Variants or analogs may be prepared by the deletion of a portion of the coding sequence, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. See, for example, Sambrook *et al.*, supra; DNA Cloning, Vols. I and II, supra; Nucleic Acid Hybridization, supra.

"Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequences. A coding sequence may be operably linked to control sequences that direct the transcription of the polynucleotide whereby said polynucleotide is expressed in a host cell.

The term "transfection" refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, or the molecular form of the polynucleotide that is inserted. The insertion of a polynucleotide per se and the insertion of a plasmid or vector comprised of the exogenous polynucleotide are included. The exogenous polynucleotide may be directly transcribed and translated by the cell, maintained as a nonintegrated vector, for example, a plasmid, or alternatively, may be stably integrated into the host genome. "Transfection" generally is used in reference to a eukaryotic cell while the term "transformation" is used to refer to the insertion of a polynucleotide into a prokaryotic cell. "Transformation" of a eukaryotic cell also may refer to the formation of a cancerous or tumorigenic state.

The term "isolated," when referring to a polynucleotide or a polypeptide, intends that the indicated molecule is present in the substantial absence of other similar biological macromolecules. The term "isolated" as used herein means that at least 75 wt.%, more preferably at least 85 wt.%, more preferably still at least 95 wt.%, and most preferably at least 98 wt.% of a composition is the isolated polynucleotide or polypeptide. An "isolated polynucleotide" that encodes a particular polypeptide refers to a polynucleotide that is substantially free of other nucleic acid molecules that do not

encode the subject polypeptide; however, the molecule may include functionally and/or structurally conservative mutations as defined herein.

A "test sample" as used herein intends a component of an individual's body which is a source of a $P2X_2$ receptor. These test samples include biological samples which can be evaluated by the methods of the present invention described herein and include body fluids such as whole blood, tissues and cell preparations.

The following single-letter amino acid abbreviations are used throughout the text:

| | | | |
|---|---|---|---|
| Alanine | A | Arginine | R |
| Asparagine | N | Aspartic acid | D |
| Cysteine | C | Glutamine | Q |
| Glutamic acid | E | Glycine | G |
| Histidine | H | Isoleucine | I |
| Leucine | L | Lysine | K |
| Methionine | M | Phenylalanine | F |
| Proline | P | Serine | S |
| Threonine | T | Tryptophan | W |
| Tyrosine | Y | Valine | V |

A human $P2X_2$ receptor, a polynucleotide encoding the variant receptor or polypeptide subunits thereof, and methods of making the receptor are provided herein. The invention includes not only the $P2X_2$ receptor but also methods for screening compounds using the receptor and cells expressing the receptor. Further, polynucleotides and antibodies which can be used in methods for detection of the receptor, as well as the reagents useful in these methods, are provided. Compounds and polynucleotides useful in regulating the receptor and its expression also are provided as disclosed hereinbelow.

In one preferred embodiment, the polynucleotide encodes a human $P2X_2$ receptor polypeptide or a protein variant thereof containing conservative amino acid substitutions.

DNA encoding the human $P2X_2$ receptor, and variants thereof, can be derived from genomic or cDNA, prepared by synthesis, or by a combination of techniques. The DNA can then be used to express the human $P2X_2$ receptor or as a template for the preparation of RNA using methods well known in the art (see, Sambrook *et al.*, supra), or as a molecular probe capable of selectively hybridizing to, and therefore detecting the presence of, other $P2X_2$-encoding nucleotide sequences.

cDNA encoding the $P2X_2$ receptor may be obtained from an appropriate DNA library. cDNA libraries may be probed using the procedure described by Grunstein *et al.* (1975) Proc. Natl. Acad. Sci. USA 73:3961. The cDNA thus obtained can then be modified and amplified using the polymerase chain reaction ("PCR") and primer sequences to obtain the DNA encoding the human $P2X_2$ receptor.

More particularly, PCR employs short oligonucleotide primers (generally 10-20 nucleotides in length) that match opposite ends of a desired sequence within the DNA molecule. The sequence between the primers need not be known. The initial template can be either RNA or DNA. If RNA is used, it is first reverse transcribed to cDNA. The cDNA is then denatured, using well-known techniques such as heat, and appropriate oligonucleotide primers are added in molar excess.

Primer extension is effected using DNA polymerase in the presence of deoxynucleotide triphosphates or nucleotide analogs. The resulting product includes the respective primers at their 5'-termini, covalently linked to the newly synthesized complements of the original strands. The replicated molecule is again denatured, hybridized with primers, and so on, until the product is sufficiently amplified. Such PCR methods are described in for example, U.S. Patent Nos. 4,965,188; 4,800,159; 4,683,202; 4,683,195; incorporated herein by reference in their entireties. The product of the PCR is cloned and the clones containing the $P2X_2$ receptor DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using a primer as a hybridization probe.

Alternatively still, the $P2X_2$ receptor DNA could be generated using an RT-PCR (reverse transcriptase - polymerase chain reaction) approach starting with human RNA. Human RNA may be obtained from cells or tissue in which the $P2X_2$ receptor is expressed, for example, brain, spinal cord, uterus or lung, using conventional methods. For example, single-stranded cDNA is synthesized from human RNA as the template using standard reverse transcriptase procedures and the cDNA is amplified using PCR. This is but one example of the generation of $P2X_2$ receptor variant from a human tissue RNA template.

Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer such as that described by Warner (1984) DNA 3:401. If desired, the synthetic strands may be labeled with $^{32}P$ by treatment with polynucleotide kinase in the presence of $^{32}P$-ATP, using standard conditions for the reaction. DNA sequences, including those isolated from genomic or cDNA libraries, may be modified by known methods which include site-directed mutagenesis as described by Zoller (1982) Nucleic Acids Res. 10:6487. Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a

double stranded DNA with DNA polymerase using, as a primer, a synthetic oligonucleotide complementary to the portion of the DNA to be modified, and having the desired modification included in its own sequence. Culture of the transformed bacteria, which contain replications of each strand of the phage, are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions suitable for hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and cloned. Alternatively, it may be necessary to identify clones by sequence analysis if there is difficulty in distinguishing the variant from wild type by hybridization. In any case, the DNA would be sequence-confirmed.

Once produced, DNA encoding the $P2X_2$ receptor may then be incorporated into a cloning vector or an expression vector for replication in a suitable host cell. Vector construction employs methods known in the art. Generally, site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions that generally are specified by the manufacturer of these commercially available enzymes. After incubation with the restriction enzyme, protein is removed by extraction and the DNA recovered by precipitation. The cleaved fragments may be separated using, for example, polyacrylamide or agarose gel electrophoresis methods, according to methods known by those of skill in the art.

Sticky end cleavage fragments may be blunt ended using *E. coli* DNA polymerase 1 (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease also may be used, resulting in the hydrolysis of any single stranded DNA portions.

Ligations are performed using standard buffer and temperature conditions using T4 DNA ligase and ATP. Alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation.

Standard vector constructions generally include specific antibiotic resistance elements. Ligation mixtures are transformed into a suitable host, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants can then be prepared according to methods known to those in the art usually following a chloramphenicol amplification as reported by Clewell *et al.* (1972) J. Bacteriol. 110:667. The DNA is isolated and analyzed usually by restriction enzyme analysis and/or sequencing. Sequencing may be by the well-known dideoxy method of Sanger *et al.* (1977) Proc. Natl. Acad. Sci. USA 74:5463) as further described by Messing *et al.* (1981) Nucleic Acid Res. 9:309, or by the method

reported by Maxam *et al.* (1980) Meth. Enzymol. 65:499. Problems with band compression, which are sometimes observed in GC rich regions, are overcome by use of, for example, T-deazoguanosine or inosine, according to the method reported by Barr *et al.* (1986) Biotechniques 4:428.

Host cells are genetically engineered with the vectors of this invention, which may be a cloning vector or an expression vector. The vector may be in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants/transfectants or amplifying the subunit-encoding polynucleotide. The culture conditions, such as temperature, pH and the like, generally are similar to those previously used with the host cell selected for expression, and will be apparent to those of skill in the art.

Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences that are compatible with the designated host are used. For example, among prokaryotic hosts, *Escherichia coli* is frequently used. Also, for example, expression control sequences for prokaryotes include but are not limited to promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts can be derived from, for example, the plasmid pBR322 that contains operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, that also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include but are not limited to the lactose operon system (Chang *et al.* (1977) Nature 198:1056), the tryptophan operon system (reported by Goeddel *et al.* (1980) Nucleic Acid Res. 8:4057) and the lambda-derived Pl promoter and N gene ribosome binding site (Shimatake *et al.* (1981) Nature 292:128), the hybrid Tac promoter (De Boer *et al.* (1983) Proc. Natl. Acad. Sci. USA 292:128) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with *E. coli*; however, other prokaryotic hosts such as strains of Bacillus or Pseudomonas may be used if desired.

Eukaryotic hosts include yeast and mammalian cells in culture systems. *Pichia pastoris, Saccharomyces cerevisiae* and *S. carlsbergensis* are commonly used yeast hosts. Yeast-compatible vectors carry markers that permit selection of successful transformants by conferring protrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast-compatible vectors may employ the 2-μ origin of replication (Broach *et al.* (1983) Meth. Enzymol. 101:307), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences that will result

in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include but are not limited to promoters for the synthesis of glycolytic enzymes, including the promoter for 3-phosphoglycerate kinase. See, for example, Hess *et al.* (1968) J. Adv. Enzyme Reg. 7:149, Holland *et al.* (1978) Biochemistry 17:4900 and Hitzeman (1980) J. Biol. Chem. 255:2073. For example, some useful control systems are those that comprise the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, or the hybrid yeast promoter ADH2/GAPDH described in Cousens *et al.* Gene (1987) 61:265-275, terminators also derived from GAPDH, and, if secretion is desired, leader sequences from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are not naturally associated in the wild-type organism.

Mammalian cell lines available as hosts for expression are known in the art and are available from depositories such as the American Type Culture Collection. These include but are not limited to HeLa cells, human embryonic kidney (HEK) cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and others. Suitable promoters for mammalian cells also are known in the art and include viral promoters such as that from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), bovine papilloma virus (BPV) and cytomegalovirus (CMV). Mammalian cells also may require terminator sequences and poly A addition sequences; enhancer sequences which increase expression also may be included, and sequences which cause amplification of the gene also may be desirable. These sequences are known in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which ensure integration of the appropriate sequences encoding the $P2X_2$ receptor into the host genome. An example of such a mammalian expression system is described in Gopalakrishnan *et al.* (1995), Eur. J. Pharmacol.-Mol. Pharmacol. 290: 237-246.

Other eukaryotic systems are also known, as are methods for introducing polynucleotides into such systems, such as amphibian cells, using standard methods such as described in Briggs *et al.* (1995) Neuropharmacol. 34:583-590 or Stühmer (1992) Meth. Enzymol. 207:319-345, insect cells using methods described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), and the like.

The baculovirus expression system can be used to generate high levels of recombinant proteins in insect host cells. This system allows for high level of protein expression, while post-translationally processing the protein in a manner similar to

mammalian cells. These expression systems use viral promoters that are activated following baculovirus infection to drive expression of cloned genes in the insect cells (O'Reilly *et al.* (1992) Baculovirus Expression Vectors: A Laboratory Manual, IRL/Oxford University Press).

Transfection may be by any known method for introducing polynucleotides into a host cell, including packaging the polynucleotide in a virus and transducing a host cell with the virus, by direct uptake of the polynucleotide by the host cell, and the like, which methods are known to those skilled in the art. The transfection procedures selected depend upon the host to be transfected and are determined by the rountineer.

The expression of the receptor may be detected by use of a radioligand selective for the receptor. However, any radioligand binding technique known in the art may be used to detect the receptor (see, for example, Winzor *et al.* (1995) Quantitative Characterization of Ligand Binding, Wiley-Liss, Inc., NY; Michel *et al.* (1997) Mol. Pharmacol. 51:524-532). Alternatively, expression can be detected by utilizing antibodies or functional measurements, *i.e.*, ATP-stimulated cellular depolarization using methods that are well known to those skilled in the art. For example, agonist-stimulated $Ca^{2+}$influx, or inhibition by antagonists of agonist-stimulated $Ca^{2+}$influx, can be measured in mammalian cells transfected with the recombinant $P2X_2$ receptor cDNA, such as COS, CHO or HEK cells. Alternatively, $Ca^{2+}$influx can be measured in cells that do not naturally express P2 receptors, for example, the 1321N1 human astrocytoma cell line, have been prepared using recombinant technology to transiently or stably express the $P2X_2$ receptor.

The $P2X_2$ polypeptide is recovered and purified from recombinant host cell cultures expressing the same by known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The human $P2X_2$ receptor polypeptide, or fragments thereof, of the present invention also may be synthesized by conventional techniques known in the art, for example, by chemical synthesis such as solid phase peptide synthesis. In general, these methods employ either solid or solution phase synthesis methods. See, for example, J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, The

Peptides: Analysis, Synthesis, Biology, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., The Peptides: Analysis, Synthesis, Biology, supra, Vol. 1, for classical solution synthesis.

In one preferred system, either the DNA or the RNA derived therefrom, each of which encode the human $P2X_2$ receptor, may be expressed by direct injection into a cell, such as a Xenopus laevis oocyte. Using this method, the functionality of the human $P2X_2$ receptor encoded by the DNA or the mRNA can be evaluated as follows. A receptor-encoding polynucleotide is injected into an oocyte for translation into a functional receptor subunit. The function of the expressed variant human $P2X_2$ receptor can be assessed in the oocyte by a variety of techniques including electrophysiological techniques such as voltage-clamping, and the like.

Receptors expressed in a recombinant host cell may be used to identify compounds that modulate $P2X_2$ activity. In this regard, the specificity of the binding of a compound showing affinity for the receptor is demonstrated by measuring the affinity of the compound for cells expressing the receptor or membranes from these cells. This may be done by measuring specific binding of labeled (for example, radioactive) compound to the cells, cell membranes or isolated receptor, or by measuring the ability of the compound to displace the specific binding of a standard labeled ligand. See, Michel *et al.*, supra. Expression of variant receptors and screening for compounds that bind to, or inhibit the binding of labeled ligand to these cells or membranes, provide a method for rapid selection of compounds with high affinity for the receptor. These compounds may be agonists, antagonists or modulators of the receptor.

Expressed receptors also may be used to screen for compounds that modulate $P2X_2$ receptor activity. One method for identifying compounds that modulate $P2X_2$ activity, comprises providing a cell that expresses a human $P2X_2$ receptor polypeptide, combining a test compound with the cell and measuring the effect of the test compound on the $P2X_2$ receptor activity. The cell may be a bacterial cell, a mammalian cell, a yeast cell, an amphibian cell, an insect or any other cell expressing the receptor. Preferably, the cell is a mammalian cell or an amphibian cell. Thus, for example, a test compound is evaluated for its ability to elicit an appropriate response, for example, the stimulation of cellular depolarization, or for its ability to modulate the response to an agonist or antagonist.

Additionally, compounds capable of modulating $P2X_2$ receptors are considered potential therapeutic agents in several disorders including, without limitation, central

nervous system or peripheral nervous system conditions, for example, epilepsy, pain, depression, neurodegenerative diseases, and the like, and in disorders of skeletal muscle such as neuromuscular diseases.

In addition, the DNA, or RNA derived therefrom, can be used to design oligonucleotide probes for DNAs that express $P2X_2$ receptors. As used herein, the term "probe" refers to a structure comprised of a polynucleotide, as defined above, which contains a nucleic acid sequence complementary to a nucleic acid sequence present in a target polynucleotide. The polynucleotide regions of probes may be composed of DNA, and/or RNA, and/or synthetic nucleotide analogs. Such probes could be useful in *in vitro* hybridization assays to distinguish $P2X_2$ variant from wild-type message, with the proviso that it may be difficult to design a method capable of making such a distinction given the small differences that may exist between sequences coding the wild-type and a variant $P2X_2$ receptor. Alternatively, a PCR-based assay could be used to amplify the sample RNA or DNA for sequence analysis.

Furthermore, the $P2X_2$ polypeptide or fragment(s) thereof can be used to prepare monoclonal antibodies using techniques that are well known in the art. The $P2X_2$ receptor or relevant fragments can be obtained using the recombinant technology outlined below, *i.e.*, a recombinant cell that expresses the receptor or fragments can be cultured to produce quantities of the receptor or fragment that can be recovered and isolated. Alternatively, the $P2X_2$ polypeptide or fragment(s) thereof can be synthesized using conventional polypeptide synthetic techniques as known in the art. Monoclonal antibodies that display specificity and selectivity for the $P2X_2$ polypeptide can be labeled with a measurable and detectable moiety, for example, a fluorescent moiety, radiolabels, enzymes, chemiluminescent labels and the like, and used in *in vitro* assays. It is theorized that such antibodies could be used to identify wild-type or variant $P2X_2$ receptor polypeptides for immuno-diagnostic purposes. For example, antibodies have been generated to detect amyloid b1-40 v. 1-42 in brain tissue (Wisniewski *et al.* (1996) Biochem. J. 313:575-580; also see, Suzuki *et al.* (1994) Science 264:1336-1340; Gravina *et al.* (1995) J. Biol. Chem. 270:7013- 7016; and Turnet *et al.* (1996) J. Biol. Chem. 271:8966-8970).

Therapeutic Indications for Modulators of the Human $P2X_2$ Receptor

Activation of the $P2X_2$ receptor by ATP and other nucleotides regulates ion gradients across the cell membrane, modulates the cytosolic concentrations of cations, including Ca2+, Na+ and K+, and has a role in the regulation of cell membrane potential which in turn has specific physiological effects.

Pain

The rat $P2X_2$ receptor is expressed in the spinal cord, and in the nodose and dorsal root ganglia (Brake *et al.*, Nature 371:519-523 (1994)), a distribution consistent with a role in pain transmission. Specifically, the $P2X_2$ receptor subunit forms functional channels when expressed alone, and it can also form a functional heteromultimeric channel that has properties similar to currents seen in native sensory channels when co-expressed with the $P2X_3$ receptor, another P2X receptor which is expressed in sensory neurons (Lewis *et al.*, Nature 377:432-435 (1995)). Evidenced from studies in rat nodose ganglia indicate that both $P2X_2/P2X_3$ heteromeric channels and $P2X_2$ homomeric channels contribute to ATP currents (Virginio *et al.*, J. Physiol (Lone) 510:27-35 (1998); Thomas, *et al.*, J. Physiol (Lond) 509 (Pt 2):411-417 (1998)). ATP, which activates $P2X_2$ and $P2X_2/P2X_3$ receptors, functions as an excitatory neurotransmitter in the spinal cord dorsal horn and in primary afferents from sensory ganglia (Holton and Holton, J. Physiol. (Lond) 126:124-140 (1954)). ATP-induced activation of P2X receptors on dorsal root ganglion nerve terminals in the spinal cord stimulates the release of glutamate, a key neurotransmitter involved in nociceptive signaling (Gu and MacDermott, Nature 389:749-753 (1997)). Thus, ATP released from damaged cells evokes pain by activating $P2X_2$ or $P2X_2/P2X_3$ receptors on nociceptive nerve endings or sensory nerves. This is consistent with the induction of pain by intradermally applied ATP in the human blister-base model (Bleehen, Br J. Pharmacol 62:573-577 (1978)), and with reports that P2X receptor antagonists are analgesic in animal models (Driessen and Starke, Naunyn Schmiederbergs Arch Pharmacol 350:618-625 (1994)). This evidence clearly suggests that $P2X_2$ functions in nociception, and that modulators of the human $P2X_2$ receptor are useful as analgesics.

Thus, compounds which block or inhibit activation of $P2X_2$ receptors serve to block the pain stimulus. Antagonists to compounds which normally activate the $P2X_2$ receptor, such as ATP, could successfully block the transmission of pain.

Diseases of the Neuroendocrine System

Extracellular ATP induces secretion of hormones, including prolactin and leuteinizing hormone, from cells of the pituitary gland (Chen *et al.*, Proc Natl Acad Sci USA 92:5219-5223 (1995); Nunez *et al*, Am J. Physiol 272:E1117-E1123 (1997)). (Carew *et al.*, Cell Calcium 16:227-235 (1994)) (Villalobos *et al.*, Am J Physiol 273:C1963-C1971 (1997)). In addition, since ATP is co-released with hormones such as insulin, prolactin, and leuteinizing hormone, as well as with catecholamines from adrenal chromaffin cells, it may act as a paracrine regulator of hormone release in

these tissues (Chen *et al.*, Proc Natl Acad Sci USA 92:5219:5223 (1995); Tomic *et al.*, J Biol Chem 271:21200-21208 (1996); Nunez *et al.*, Am J Physiol 272:E1117-E1123 (1997)) (Leitner *et al.*, Endocrinology 96:662-677 (1975)); Hollins and Ikeda, J Neurophysiol 78:3069-3076 (1997)). The human $P2X_2$ receptor has been found in neuroendocrine tissue and, specifically, the human $P2X_2$ receptor cDNAs was cloned from pituitary tissue RNA. In addition, the $P2X_2$ receptor RNA and protein have been detected in rat pituitary tissue (Brake *et al.*, Nature 371:519-523 (1994)) (Housley *et al.*, Biochem Biophys Res Commun 212:501-508 (1995); Tomic *et al.*, J Biol Chem 271:21200-21208 (1996); Vulchanova *et al.*, Proc Natl Acad Sci USA 93:8063-8067 (1996)). Clearly, the $P2X_2$ receptor is involved in hormone secretion via activation by ATP. Thus, an agonist or antagonist to ATP would be effective in modulating hormone release. Thus, pharmaceutical agents that act on the $P2X_2$ receptor may be useful to modulate hormonal secretion from this gland.

Auditory and Vestibular Disorders

Extracellular ATP acts as a stimulus for neurons and epithelial cells of the inner ear (Housley, Mol Neurobial 16:21-48 (1998)). Perfusion of ATP into the guinea pig cochlear perilymphatic compartment inhibits auditory parameters such as auditory-nerve compound action potential and sound tranduction current across the apical surface of sensory hair cells. (Bobbin and Thompson, Ann Otol Rhinol Laryngol 87:185-190 (1978)). Perfusion of ATP into the cochlear endolymph also inhibits sensory current transduction and endocochlear potential, and these effects are blocked by the P2 receptor antagonists suramin and reactive blue 2 (Munoz *et al.*, Hear Res 90:119-125 (1995)). Suramin also blocks the decline in quadratic electrophysiological and mechanical coupling of the organ of Corti which occurs during continuous sound stimulation, suggesting that P2 activation plays a role in this event (Kujawa *et al.*, Hear Res 78:181-188 (1994); (Housley, Mol Neurogiol 16:21-48 (1998)). ATP also affects vestibular system function. ATP stimulates vestibular afferent nerve discharge, and these responses are blocked by the P2 antagonist suramin and reactive blue 2 (Aubert *et al.*, Neuroscience 62-963-974 (1994); Aubert *et al.*, Neuroscience 64:1153-1160 (1995)). Autoradiographic binding studies using ATP analogs indicate the presence of P2 receptors on auditory tissues (Mockett *et al.*, Hear Res 84:177-193 (1995)). $P2X_2$ receptor messenger RNA has been localized in tissues of the rat auditory system. Several message variants for this receptor have been found in various vestibular and auditory tissues, including the cochlea, spiral ganglia, Dieter's cells, christa ampullaris, and the organ of Corti (Glowatzki *et al.*, Proc R Soc Lond B Biol Sci 262:141-147 (1995); Housley *et al.*, Biochem Biophys Res

Commun 212:501-508 (1995); Salih *et al.*, Neuroreport 9:279-282 (1998); Chen and Bobbin, Br J Pharmacol 124:337-344 (1998); Housley *et al.*, J Comp Neurol 393:403-414 (1998)). Evidence of the expression of $P2X_2$ receptors in those tissues of the auditory and vestibular systems which are functionally modulated by ATP indicates a role for this receptor in auditory and vestibular function. Altered function of P2 receptors in the ear have pathological implications, as exposure to noise has been shown to alter the response of outer hair cells to ATP (Chen *et al.*, Hear Res 88:215-221(1995)), and $P2X_2$ receptor modulators may have utility in disorders of auditory and vestibular function. Thus, ATP agonists and antagonists have effects on modulation of the $P2X_2$ receptor, in auditory and vestibular functions.

Other

ATP is a potent neurotransmitter in neurons of the gastrointestinal tract, and ATP-mediated signals from enteric neurons appears to be characteristic of $P2X_2$ receptors (Zhou and Galligan, J Physiol (Lond) 496 (Pt 3):719-729 (1996)). Additionally, the discovery of the human $P2X_2$ EST from a library derived from colon tissue suggests that this receptor plays a role in gastrointestinal function. $P2X_2$ is also expressed in vascular smooth muscle tissue, where ATP has been shown to influence vascular tone (Nori *et al.*, J. Vasc Res 35:179-185 (1998)) (Kennedy *et al.*, Eur J Pharmacol 107:161-168 (1985)).

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

## Example 1

## Identification of a Human cDNA Sequence Likely to Encode $P2X_2$ Polypeptide

The predicted amino acid sequence of the rat $P2X_2$ receptor (Genbank accession number 1352688) was used to search for human DNA sequences which would code for similar polypeptides. The TBLASTN database search tool (Altschul (1993) J. Mol. Evol. 36:290-300) was used, which allows querying nucleotide databases with a protein sequence by dynamically translating the DNA sequences into all 6 possible reading frames. A search of the Lifeseq database (Incyte Pharmaceuticals, Inc., Palo Alto California, CA) revealed a partial sequence of cDNA clone derived from human fetal colon tissue which encoded a polypeptide having a high degree of homology to a region of the rat $P2X_2$ receptor. The database entry for this sequence is shown in Figure 1 and SEQ. ID NO:1.

The position of this sequence with respect to that of the rat $P2X_2$ sequence predicted that this cDNA clone would only contain a partial coding sequence for the receptor. The cDNA clone was ordered and the clone was fully sequenced as shown in Figure 2 and SEQ ID NO:2. Note that in Figure 2 the underlined sequence denotes overlap with the original database entry.

Primers were designed to the non-coding sequence of this cDNA to enable 5' RACE procedures in an attempt to identify the missing coding sequence, shown in Figure 3 and SEQ.ID. NOS:3-7. Using poly A plus RNA derived from human pituitary tissue, 5' RACE reactions were performed using a commercially available system (GibcoBRL, Gaithersburg, MD). A product of approximately 600 bp was cloned and sequenced, shown in Figure 4 and SEQ ID NO:8. This product was found to contain additional sequence information for an open reading frame with homology to the P2X receptors, but did not extend to what would be the predicted initiation codon of an intact receptor cDNA.

A pair of primers were designed and synthesized based on the sequence compiled from Incyte clone 1310493 and the RACE product, and are shown in Figure 5. These primers were sent to Genome Systems (St. Louis, MO) and used in PCR reactions to probe a P1 bacteriophage library of human genomic DNA. Two clones were identified and obtained from Genome systems. The human $P2X_2$ gene contained in clone 18860 was sequenced both directly and after subcloning into the vector pBluescript II SK+.

## Example 2

## Isolation of Human cDNAs Encoding Novel P2 Receptors

Using information on the sequence surrounding the predicted initiation and termination codons of the human $P2X_2$ message, oligonucleotide primers were designed and synthesized to enable RT-PCR of the intact open reading frame of the mRNA. The sequence of these primers, $hP2X_2$ 5' and $hP2X_2$ 3', are shown in Figure 6. The primers were used to amplify the open reading frames of human $P2X_2$ receptors in reverse transcription- PCR reactions as follows: Poly A+ RNA (1 microgram) derived from pituitary gland tissue (Clontech, Inc. Palo Alto, CA) and 10 picomoles oligo dT primer were combined in a final volume of 12 µl $dH_2O$. This mixture was heated to 70°C for 10 min. and chilled on ice for 1 min. The following components were added: 2 µl 10X PCR buffer (200 mM Tris-HCl pH 8.4, 500mM KCl), 2 µl 25 mM $MgCl_2$, 1 µl 10mM dNTP mix, and 2 µl 0.1M dithiothreitol. The reaction was equilibrated to 42°C for 2 minutes after which 1 µl (200 units)

Superscript II reverse transcriptase was added and incubation continued at 42°C for 50 minutes. The reaction was terminated by incubation at 70°C for 15 min. and chilled on ice. Rnase H (1 µl; 2 units) was added and the mixture was incubated for 20 minutes at 37°C, then stored on ice.

A proofreading thermostable polymerase (Cloned Pfu DNA Polymerase, Stratagene Inc. La Jolla, CA) was used in the amplification to ensure high-fidelity amplification. The reaction mixture consisted of: 2 µl cDNA, 5 µl 10x cloned Pfu polymerase reaction buffer (200 mM Tris-HCl (pH 8.8), 100mMKCl, 100mM$(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% Triton X-100, 1 mg/ml nuclease-free bovine serum albumin), 1 µl dNTP mix, 1µl (10picomoles) 5'hP2$X_2$ primer, 1µl (10 picomoles) 3'hP2$X_2$ primer, and 39 µl $dH_2O$. The reaction was heated to 95°C for 1 min., then held at 80°C for 2 min., during which 1 µl (2.5 units) cloned Pfu polymerase was added. The reaction was cycled 35 times under these conditions; 94°C for 15 sec., 60°C for 20 sec., and 72°C for 5 minutes. After cycling, the reaction was incubated for 10 minutes at 70°C. The reaction products were separated on a 0.8 % agarose gel and products of approximately 1.5 kilobases were excised and purified via the Qiaquick gel purification system (Qiagen, Inc., Chatsworth, CA). The DNA was eluted with 50 µl $dH_2O$, lyophilized and resuspended in 10 µl $dH_2O$. The DNA was eluted with 50 µl $dH_2O$, lyophilized and resuspended in 15 µl $dH_2O$. Three microliters of the purified PCR product was used in a ligation reaction using the pCRscript cloning system (Stratagene) which also included: 0.5µl (5 ng) of the pCRscript Amp SK(+) vector, 1µl of pCRscript 10x Reaction Buffer, 0.5 µl of 10mM ATP, 1µl (5 units) Srf I restriction enzyme, 1µl (4 units) T4 DNA ligase, and 3 µl $dH_2O$. The reaction was incubated at room temperature for one hour, then at 65°C for 10 minutes. One microliter of this reaction was used to transform ultracompetent DH-5-α(Gibco BRL) as per standard manufacturer's protocols. Resulting clones were screened by restriction analysis and sequenced using fluorescent dye-terminator reagents (Prism, Perkin Elmer Applied Biosystems) and an Applied Biosystems 310 DNA sequencer. Three species of cDNAs containing intact open reading frames from the predicted initiation to termination codons were isolated (Figure 7, hP2$X_{2b}$, c, d). Based on structural similarity to the rat P2$X_2$ receptor, a fourth species, (hP2$X_{2a}$, Figure 7a) was created by joining nucleotides 1-666 (using adenine of the initiation codon as nucleotide #1) of hP2$X_{2d}$ with nucleotides 595-1349 of hP2$X_{2c}$. The predicted polypeptides encoded by these cDNAs are shown in Figure 8. An alignment of the predicted amino acid sequences are shown in Figure 9.

## Example 3

## Expression and Electrophysiological Analysis of Recombinant $P2X_2$ Receptors in Xenopus Oocytes

To assess function of the human $P2X_2$ receptors, RNA was synthesized from the clones using the $T_7$ bacterial promoter present on the pCRscript vector and reagents from Ambion (Message Machine; Ambion, Inc., Austin Tx.).

### 1. Preparation and injection of oocytes

Adult female frogs (Xenopus laevis) were anesthetized with 0.2% tricaine before surgery. During surgery, sections of one ovary were removed and oocytes were denuded of overlying follicle cells by agitation for 1-2 hours in 2 mg/ml collagenase (Sigma type IA) in low-$Ca^{2+}$ Barth's solution containing (in mM): 88 NaCl, 2.5 KCl, 1.0 $MgCl_2$ 10 Na-HEPES (pH 7.4) plus 100 µg/ml gentamicin. Selection of stage V and VI oocytes was begun after approximately 50% of the cells were denuded. Cytoplasmic injections of 50 ng $hP2X_{2a-d}$ RNA were performed on denuded oocytes using a glass microelectrode. Only one receptor subtype RNA was injected per cell. Oocytes were used for recording 1-2 days after injection and were maintained at 16-19°C in normal Barth's solution (incubation medium in mM): 90 NaCl, 1.0 KCl, 0.66 $NaNO_3$, 0.74 $CaCl_2$, 0.82 $MgCl_2$, 2.4 $NaHCO_3$, 2.5 Na-pyruvate, 10 Na-HEPES (pH 7.4) plus 100 µg/ml gentamicin.

### 2. Recording solutions and chemicals

The standard recording solution contained (in mM): 96 NaCl, 2.0 KCl, 1.8 $BaCl_2$, 1.0 $MgCl_2$, 5.0 Na-pyruvate, and 5.0 Na-HEPES (pH 7.4). $BaCl_2$ was replaced with $CaCl_2$ (1 mM) in some experiments without significant effects on the pharmacological properties of the receptors. All oocyte solutions were diluted in distilled $H_20$ from 10X stock solutions. Concentrated stocks of agonists and antagonists were made in distilled $H_20$ and then serially diluted in recording solution to desired final concentrations. All chemicals and agonists (ATP and α,βme-ATP) were obtained from Sigma Chemical Company.

3. Electrophysiological recordings

Transmembrane currents were recorded using two-electrode voltage-clamp techniques with an Axoclamp-2A amplifier, and were collected and analyzed using pCLAMP software (Axon Instruments). Electrodes (1.5 - 2.0 MΩ) were filled with 120 mM KCl. Responses to ATP and α,βme-ATP were routinely recorded at room temperature while the oocyte membrane was voltage-clamped at -60 mV. Agonists were applied using a computer-controlled small diameter drug application pipette positioned close to the oocyte in the perfusion chamber. Application duration typically lasted 5-10 sec. The peak amplitude of the ATP-activated inward current was used for determining $EC_{50}$ values.

4. Results

$hP2X_{2a}$ and $hP2X_{2b}$ receptors - Transient external application of ATP to oocytes expressing $hP2X_{2a}$ or $hP2X_{2b}$ receptors produced a concentration-dependent increase in net inward current (Figure 10, panels A and B). Peak inward current increased with increasing ATP concentrations, consistent with an increase in probability of agonist binding, and therefore receptor activation. Concentration-response curves for four $hP2X_{2a}$ cells revealed a mean ATP $EC_{50}$ of 16 μM, and a Hill coefficient ($n_H$) of 1.5. Concentration-response curves for three $hP2X_{2b}$ cells revealed a mean ATP $EC_{50}$ of 20 μM, and a $n_H$ of 1.5. Both receptor subtypes exhibited reversible non-desensitizing response kinetics.

Application of another P2X receptor agonist, αβMethylene-ATP (αβMe-ATP) had no effect on $hP2X_{2a}$ or $hP2X_{2b}$ receptors at a concentration of 100 μM.

5. $hP2X_{2c}$ and $hP2X_{2d}$ receptors

Transient external application of ATP (30 μM) to oocytes injected with $hP2X_{2d}$ or $hP2X_{2d}$ RNA had no effect (Figure 10, panels C and D).

6. Conclusions

Using an electrophysiological approach to analyze $hP2X_{2a-d}$ receptor function, we have shown that two receptor subtypes ($hP2X_{2a}$ and $hP2X_{2b}$) can be selectively activated by ATP, but not αβMe-ATP. These responses are also non-desensitizing. The $hP2X_{2c}$ and $hP2X_{2d}$ subtypes expressed alone did not respond to ATP. These data support the formation of functional homomeric recombinant $hP2X_{2a}$ and $hP2X_{2b}$ ion channel receptors.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding a human $P2X_2$ receptor polypeptide or a degenerate variant thereof.

2. A polynucleotide according to Claim 1, wherein the polynucleotide is a polydeoxyribonucleotide (DNA).

3. A polynucleotide according to Claim 1, wherein the polynucleotide is a polyribonucleotide (RNA).

4. A polynucleotide according to Claim 2, wherein the DNA is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

5. A host cell comprising a polynucleotide according to Claim 1 or Claim 4.

6. A host cell according to Claim 5, wherein said cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell and an amphibian cell.

7. A host cell according to Claim 6, wherein the cell is an amphibian cell.

8. A host cell according to Claim 6, wherein the cell is a mammalian cell.

9. An expression vector comprising a polynucleotide according to Claim 1 operably linked to control sequences that direct the transcription of the polynucleotide, whereby the polynucleotide is expressed in a host cell.

10. An expression vector according to Claim 9, wherein the human $P2X_2$ receptor polypeptide is selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20.

11. A host cell comprising an expression vector according to Claim 9.

12. A host cell according to Claim 11, wherein the cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell and an amphibian cell.

13. A host cell according to Claim 12, wherein the cell is an amphibian cell.

14. A host cell according to Claim 12, wherein the cell is a mammalian cell.

15. A host cell comprising the expression vector of Claim 10.

16. A host cell according to Claim 15, wherein the cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell and an amphibian cell.

17. A host cell according to Claim 16, wherein the cell is an amphibian cell.

18. A host cell according to Claim 16, wherein the cell is a mammalian cell.

19. A method for producing a human $P2X_2$ receptor polypeptide, the method comprising the steps of:

(a) culturing a host cell containing an expression vector under conditions that allow the production of the polypeptide, wherein said expression vector comprises a polynucleotide encoding a human $P2X_2$ receptor polynucleotide, or a degenerate variant thereto, which is operably linked to control sequences that direct the transcription of the polynucleotide; and

(b) recovering the polypeptide.

20. A method for producing a human $P2X_2$ receptor polypeptide, the method comprising the steps of:

(a) culturing a host cell containing an expression vector under conditions that allow the production of the polypeptide, wherein said expression vector comprises a polynucleotide operably linked to control sequences that direct the transcription of the polynucleotide, wherein said polynucleotide encodes for a human $P2X_2$ receptor polypeptide selected from the group consisting of SEQ.ID NO:17, SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO:20; and

(b) recovering the polypeptide.

21. An isolated and purified human $P2X_2$ receptor polypeptide, wherein the human $P2X_2$ receptor comprises the amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20.

22. A method for identifying compounds that modulate $P2X_2$ receptor activity, the method comprising the steps of:

(a) providing a cell that expresses a $P2X_2$ receptor comprising a human $P2X_2$ polypeptide;

(b) mixing a test compound with the $P2X_2$ receptor; and

(c) measuring either

(i) the effect of the test compound on the activation of the P2X receptor or the cell expressing the $P2X_2$ receptor, or

(ii) the binding of the test compound to the cell or the $P2X_2$ receptor.

23. A method according to Claim 22, wherein the host cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell and an amphibian cell.

24. A method according to Claim 22, wherein said measurement of step (c) (ii) is performed by measuring a signal generated by a detectable moiety.

25. A method according to Claim 24, wherein said detectable moiety is selected from the group consisting of a fluorescent label, a radiolabel, a chemiluminescent label and an enzyme.

26. A method according to Claim 22, wherein said measurement of step (c) (i) is performed by measuring a signal generated by a radiolabeled ion, a chromogenic reagent, a fluorescent probe or an electrical current.

27. A method according to Claim 23, wherein the host cell is a mammalian cell.

28. A method according to Claim 23, wherein the host cell is an amphibian cell.

29. A method according to Claim 22, wherein the human $P2X_2$ polypeptide is selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20.

30. A method for detecting a target polynucleotide of a $P2X_2$ receptor in a test sample, the method comprising the steps of:

(a) contacting the target polynucleotide with at least one human $P2X_2$ receptor-specific polynucleotide probe or a complement thereof to form a target-probe complex; and

(b) detecting the presence of the target-probe complex in the test sample.

31. A method for detecting cDNA of human $P2X_2$ receptor mRNA in a test sample, the method comprising the steps of:

(a) performing reverse transcription in order to produce cDNA;

(b) amplifying the cDNA obtained from step (a); and

(c) detecting the presence of the human $P2X_2$ receptor in the test sample.

32. A method according to Claim 31, wherein said detection step (c) comprises utilizing a detectable moiety capable of generating a measurable signal.

33. A purified polynucleotide or a fragment thereof derived from human $P2X_2$ receptor and capable of selectively hybridizing to a nucleic acid encoding a human $P2X_2$ receptor polypeptide, wherein said polynucleotide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16,or a portion thereof.

34. A purified polynucleotide according to Claim 33, wherein the polynucleotide is produced by recombinant techniques.

35. A polypeptide encoded by human $P2X_2$ receptor polynucleotide wherein said polypeptide is selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20 or a portion thereof.

36. A polypeptide according to Claim 35 produced by recombinant techniques.

37. A polypeptide according to Claim 35 produced by synthetic techniques.

38. A monoclonal antibody which specifically binds to human $P2X_2$ receptor comprising the amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, or an immunoreactive fragment thereof.

39. A method for detecting human $P2X_2$ receptor in a test sample, the method comprising the steps of:

(a) contacting the test sample with an antibody or a fragment thereof which specifically binds to the human $P2X_2$ receptor, for a time and under conditions sufficient for the formation of a resultant complex; and

(b) detecting the resultant complex containing the antibody,

wherein said antibody specifically binds to human $P2X_2$ receptor amino acid comprising the amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, or a fragment thereof.

40. A therapeutic method for relieving pain comprising:

(a) presenting an individual afflicted with pain; and

(b) administering to said individual an effective amount of a $P2X_2$ agonistic compound.

41. A therapeutic method for treating neuroendocrine disorders comprising:

(a) presenting an individual afflicted with a neuroendocrine disorder, and

(b) administering to said individual an effective amount of a a $P2X_2$ agonistic compound.

42. A therapeutic method for treating auditory and vestibalar disorders comprising:

(a) presenting an individual afflicted with a disorder selected from the group consisting of auditory disorders and vestibular disorders; and

(b) administering to said individual an effective amount of a $P2X_2$ agonistic compound.

43. A therapeutic method for treating disorders of the gastrointestinal tract comprising:

(a) presenting an individual afflicted with a gastrointestinal disorder; and

(b) administering to said individual an effective amount of a $P2X_2$ agonistic compound.

44. A method of determining whether a compound is an agonist or antagonist to $P2X_2$ receptors, comprising:

(a) contacting a mammalian cell having the $P2X_2$ receptor expressed on its surface with said compound;

(b) determining whether a biological effect is produced from the interaction of said cell and said compound; and

(c) determining whether said compound is an agonist or antagonist.

45. A method for determining whether a ligand binds to a $P2X_2$ receptor comprising:

(a) contacting a mammalian cell having the $P2X_2$ receptor expressed on its surface with a ligand;

(b) detecting the presence of the ligand; and

(c) determining whether the receptor binds to the $P2X_2$ receptors.

46. A therapeutic method for relieving pain comprising:

(a) presenting an individual afflicted with pain; and

(b) administering to said individual an effective amount of a $P2X_2$ antagonistic compound.

47. A therapeutic method for treating neuroendocrine disorders comprising:

(a) presenting an individual afflicted with a neuroendocrine disorder, and

(b) administering to said individual an effective amount of a $P2X_2$ antagonistic compound.

48. A therapeutic method for treating auditory and vestibular disorders comprising:

(a) presenting an individual afflicted with a disorder selected from the group consisting of auditory disorders and vestibular disorders; and

(b) administering to said individual an effective amount of a $P2X_2$ antagonistic compound.

49. A therapeutic method for treating disorders of the gastrointestinal tract comprising:

(a) presenting an individual afflicted with a gastrointestinal disorder, and

(b) administering to said individual an effective amount of a $P2X_2$ antagonistic compound.

CTCATCAAGGCCTACGGGATCCGCATTGACGTCATTGTGCATGGACAGGCCGGGAAGTTCAGC
CTGATTCCCACCATTATTAATCTGGCCACAGCTCTGACTTCCGTCGGGGTGGGCTCCTTCCTG
TGCGACTGGATCTTGCTAACATTCATGAACAAAAACAAGGTCTACAGCCATAAGAAATTTGAC
AAGGTGTGTACGCCGAGCCACCCCTCAGGTAG

FIG.1

GCTCATCAAGGCCTACGGGATCCGCATTGACGTCATTGTGCATGGACAGGCCGGGAAGTTCAG
CCTGATTCCCACCATTATTAATCTGGCCACAGCTCTGACTTCCGTCGGGGTGGGCTCCTTCCT
GTGCGACTGGATCTTGCTAACATTCATGAACAAAAACAAGGTCTACAGCCATAAGAAATTTGA
CAAGGTGTGTACGCCGAGCCACCCCTCAGGTAGCTGGCCTGTGACCCTTGCCCGTGTATTGGG
CCAGGCCCCTCCCGAACCCGGCCACCGCTCCGAGGACCAGCACCCCAGCCCTCCATCAGGCCA
GGAGGGCCAACAAGGGGCAGAGTGTGGCCCAGCCTTCCCGCCCCTGCGGCCTTGCCCCATCTC
TGCCCCTTCTGAGCAGATGGTGGACACTCCTGCCTCCGAGCCTGCCCAAGCCTCCACACCCAC
AGACCCCAAAGGTTTGGCTCAACTCTGAGCTCCTTTCCATCTCACTGGACTGCAGACCCGGCC
TGGTGGGGCCAGAGAGTCCCCAGCTAGGGACCTGCACGTGGACGTGGGCACCTCAGTAGCGGA
GCATCTCCACGAAACGGGGCACCACAGGATCCCTGTGCAAGGGCTGGGGGCACGCTCTGGCCC
CAGGCTTGTGCCCCACCCTGGCATACAGCCCCTGACACCTCCTCCCCAGCTGGTCCCTACAGG
GCTGCTCACTTCCCATCACCTCTCACAGCCACCTGGAACCCAAGCCAGCTGAGCTCTGAGGGG
CTCTGCTCCCGGTCTTGGGCCCTGGGAACCCCACCCCACCCCACCCCACAGGCGTTGTAACCT
CGAATCTGCCCAGACTCTTCCCTTAGAAGTCACAACATACTCAGTCCAATAAACCTGTGAGCA
GAAAAAAAAAAAAAAAAAGGGCGGCCGC

FIG.2

GSP 1 ATGAATGTTAGCAAGATCCA

FIG. 3A

GSP 2 CAUCAUCAUCAUCACCCCGACGGAAGTCAGAG

FIG. 3B

GSP3 CCTGTCCATGCACAATGACG

FIG. 3C

Anchor Primer

CUACUACUACUAGGCCACGCGTCGACTAGTACGGGNNGGGNNGGGNNG

FIG. 3D

Universal Amplification Primer

CUACUACUACUAGGCCACGCGTCGACTAGTAC

FIG. 3E

GAATTCGGCTTCTACTACTAGGCCACGCGTCGACTAGTACGGGGGGGGGGGGGGGCC

CCGGTGGAAGATGGGGCCTCTGTCAGCCAATTTCTGGGTACGATGGCCCCCAAATTTCGCGATC

CTCATCAAGAACAGCATCCATTACCCCCAAATTCCACTTCTCCAAGGGCAACATCGCCGACCGC

ACAGACGGGTACCTGAAGCGCTGCACGTTCCACGAGGCCTCCGACCTTTACTGCCCCATCTTC

AAGCTGGGCTTTATCGTGGAGAAGGCTGGGGAGAGCTTCACAGAGCTCGCACACAAGGGTGGT

GTCATCGGGGTCATTATCAACTGGGACTGTGACCTGGACCTGCCTGCATCGGAGTGCAACCCC

AAGTACTCCTTCCGGAGGCTTGACCCCAAGCACGTGCCTGCCTCGTCAGGCTACAACTTCAGG

TTTGCCAAATACTACAAGATCAATGGCACCACCACCCGCAGCTCATCAAGGCCTACGGGATCC

GCATTGACGTCATTGTGCATGGACAGG

FIG.4

X2-539F TCCTTCCTGTGCGACTGGATCTTG

FIG. 5A

X2-869R CAAACCTTTGGGGTCTGTGGGTG

FIG. 5B

hP2X25' CCACCATGGCCGCCGCCCAGCCCAAGTA

FIG. 6A

hP2X23' GGAAAGGAGCTCAGAGTTGAGCCAAACC

FIG. 6B

hP2x2a

CCACCATGGCCGCCGCCCAGCCCAAGTACCCCGCCGGGGCGACCGCCCGGCGCCTGGCCCGGG
GCTGCTGGTCCGCCCTCTGGGACTACGAGACGCCCAAGGTGATCGTGGTGAGGAACCGGCGCC
TGGGGGTCCTGTACCGCGCCGTGCAGCTGCTCATCCTGCTCTACTTCGTGTGGTACGTATTCA
TCGTGCAGAAAAGCTACCAGGAGAGCGAGACGGGCCCCGAGAGCTCCATCATCACCAAGGTCA
AGGGGATCACCACGTCCGAGCACAAAGTGTGGGACGTGGAGGAGTACGTGAAGCCCCCCGAGG
GGGGCAGCGTGTTCAGCATCATCACCAGGGTCGAGGCCACCCACTCCCAGACCCAGGGAACCT
GCCCCGAGAGCATAAGGGTCCACAACGCCACCTGCCTCTCCGACGCCGACTGCGTGGCTGGGG
AGCTGGACATGCTGGGAAACGGCCTGAGGACCGGGCGCTGTGTGCCCTATTACCAGGGGCCCT
CCAAGACCTGCGAGGTGTTCGGCTGGTGCCCGGTGGAAGATGGGGCCTCTGTCAGCCAATTTC
TGGGTACGATGGCCCCAAATTTCACCATCCTCATCAAGAACAGCATCCACTACCCCAAATTCC
ACTTCTCCAAGGGCAACATCGCCGACCGCACAGACGGGTACCTGAAGCGCTGCACGTTCCACG
AGGCCTCCGACCTCTACTGCCCCATCTTCAAGCTGGGCTTTATCGTGGAGAAGGCTGGGGAGA
GCTTCACAGAGCTCGCACACAAGGGTGGTGTCATCGGGGTCATTATCAACTGGGACTGTGACC
TGGACCTGCCTGCATCGGAGTGCAACCCCAAGTACTCCTTCCGGAGGCTTGACCCCAAGCACG
TGCCTGCCTCGTCAGGCTACAACTTCAGGTTTGCCAAATACTACAAGATCAATGGCACCACCA
CCCGCACGCTCATCAAGGCCTACGGGATCCGCATTGACGTCATTGTGCATGGACAGGCCGGGA
AGTTCAGCCTGATTCCCACCATTATTAATCTGGCCACAGCTCTGACTTCCGTCGGGGTGGGCT
CCTTCCTGTGCGACTGGATCTTGCTAACATTCATGAACAAAAACAAGGTCTACAGCCATAAGA
AATTTGACAAGGTGTGTACGCCGAGCCACCCCTCAGGTAGCTGGCCTGTGACCCTTGCCCGTG
TATTGGGCCAGGCCCCTCCCGAACCCGGCCACCGCTCCGAGGACCAGCACCCCAGCCCTCCAT
CAGGCCAGGAGGGCCAACAAGGGGCAGAGTGTGGCCCAGCCTTCCCCCCCCTGCGGCCTTGCC
CCATCTCTGCCCCTTCTGAGCAGATGGTGGACACTCCTGCCTCCGAGCCTGCCCAAGCCTCCA
CACCCACAGACCCCAAAGGTTTGGCTCAACTCTGAGCTCCTTTCCGGGCT

FIG. 7A

hP2X2b

CCACCATGGCCGCCGCCCAGCCCAAGTACCCCGCCGGGGCGACCGCCCGGCGCCTGGCCCGGG
GCTGCTGGTCCGCCCTCTGGGACTACGAGACGCCCAAGGTGATCGTGGTGAGGAACCGGCGCC
TGGGGGTCCTGTACCGCGCCGTGCAGCTGCTCATCCTGCTCTACTTCGTGTGGTACGTATTCA
TCGTGCARAAAAGCTACCAGGAGAGCGAGACGGGCCCCGAGAGCTCCATCATCACCAAGGTCA
AGGGGATCACCACGTCCGAGCACAAAGTGTGGGACGTGGAGGAGTACGTGAAGCCCCCCGAGG
GGGGCAGCGTGTTCAGCATCATCACCAGGGTCGAGGCCACCCACTCCCAGACCCAGGGAACCT
GCCCCGAGAGCATAAGGGTCCACAACGCCACCTGCCTCTCCGACGCCGACTGCGTGGCTGGGG
AGCTGGACATGCTGGGAAACGGCCTGAGGACCGGGCGCTGTGTGCCCTATTACCAGGGGCCCT
CCAAGACCTGCGAGGTGTTCGGCTGGTGCCCGGTGGAAGATGGGGCCTCTGTCAGCCAATTTC
TGGGTACGATGGCCCCAAATTTCACCATCCTCATCAAGAACAGCATCCACTACCCCAAATTCC
ACTTCTCCAAGGGCAACATCGCCGACCGCACAGACGGGTACCTGAAGCGCTGCACGTTCCACG
AGGCCTCCGACCTCTACTGCCCCATCTTCAAGCTGGGCTTTATCGTGGAGAAGGCTGGGGAGA
GCTTCACAGAGCTCGCACACAAGGGTGGTGTCATCGGGGTCATTATCAACTGGGACTGTGACC
TGGACCTGCCTGCATCGGAGTGCAACCCCAAGTACTCCTTCCGGAGGCTTGACCCCAAGCACG
TGCCTGCCTCGTCAGGCTACAACTTCAGGTTTGCCAAATACTACAAGATCAATGGCACCACCA
CCCGCACGCTCATCAAGGCCTACGGGATCCGCATTGACGTCATTGTGCATGGACAGGCCGGGA
AGTTCAGCCTGATTCCCACCATTATTAATCTGGCCACAGCTCTGACTTCCGTCGGGGTGGGCT
CCTTCCTGTGCGACTGGATCTTGCTAACATTCATGAACAAAAACAAGGTCTACAGCCATAAGA
AATTTGACAAGGTGTGTACGCCGAGCCACCCCTCAGGTAGCTGGCCTGTGACCCTTGCCCGTG
TATTGGGCCAGGCCCCTCCCGAACCCGGCCACCGCTCCGAGGACCAGCACCCCAGCCCTCCAT
CAGGCCAGGAGGGCCAACAAGGGGCAGAGTGTGGCCCAGCCTTCCCGCCCCTGCGGCCTTGCC
CCATCTCTGCCCCTTCTGAGCAGATGGTGGACACTCCTGCCTCCGAGCCTGCCCAAGCCTCCA
CACCCACAGACCCCAAAGGTTTGGCTCAACTTTGA

FIG. 7B

hP2X2c

CCACCATGGCCGCCGCCCAGCCCAAGTACCCCGCCGGGGCGACCGCCCGGCGCCTGGCCCGGG
GCTGCTGGTCCGCCCTCTGGGACTACGAGACGCCCAAGGTGATCGTGGTGAGGAACCGGCGCC
TGGGGGTCCTGTACCGCGCCGTGCAGCTGCTCATCCTGCTCTACTTCGTGTGGTACGTATTCA
TCGTGCAGAAAAGCTACCAGGAGAGCGAGACGGGCCCCGAGAGCTCCATCATCACCAAGGTCA
AGGGGATCACCACGTCCGAGCACAAAGTGTGGGACGTGGAGGAGTACGTGAAGCCCCCCGAGA
GCATAAGGGTCCACAACGCCACCTGCCTCTCCGACGCCGACTGCGTGGCTGGGGAGCTGGACA
TGCTGGGAAACGGCCTGAGGACTGGGCGCTGTGTGCCCTATTACCAGGGGCCCTCCAAGACCT
GCGAGGTGTTCGGCTGGTGCCCGGTGGAAGATGGGGCCTCTGTCAGCCAATTTCTGGGTACGA
TGGCCCCAAATTTCACCATCCTCATCAAGAACAGCATCCACTACCCCAAATTCCACTTCTCCA
AGGGCAACATCGCCGACCGCACAGACGGGTACCTGAAGCGCTGCACGTTCCACGAGGCCTCCG
ACCTCTACTGCCCCATCTTCAAGCTGGGCTTTATCGTGGAGAAGGCTGGGGAGAGCTTCACAG
AGCTCGCACACAAGGGTGGTGTCATCGGGGTCATTATCAACTGGGACTGTGACCTGGACCTGC
CTGCATCGGAGTGCAACCCCAAGTACTCCTTCCGGAGGCTTGACCCCAAGCACGTGCCTGCCT
CGTCAGGCTACAACTTCAGGTTTGCCAAATACTACAAGATCAATGGCACCACCACCCGCACGC
TCATCAAGGCCTACGGGATCCGCATTGACGTCATTGTGCATGGACAGGCCGGGAAGTTCAGCC
TGATTCCCACCATTATTAATCTGGCCACAGCTCTGACTTCCGTCGGGGTGGGCTCCTTCCTGT
GCGACTGGATCTTGCTAACATTCATGAACAAAAACAAGGTCTACAGCCATAAGAAATTTGACA
AGGTGTGTACGCCGAGCCACCCCTCAGGTAGCTGGCCTGTGACCCTTGCCCGTGTATTGGGCC
AGGCCCCTCCCGAACCCGGCCACCGCTCCGAGGACCAGCACCCCAGCCCTCCATCAGGCCAGG
AGGGCCAACAAGGGGCAGAGTGTGGCCCAGCCTTCCCGCCCCTGCGGCCTTGCCCCATCTCTG
CCCCTTCTGAGCAGATGGTGGACACTCCTGCCTCCGAGCCTGCCCAAGCCTCCACACCCACAG
ACCCCAAAGGTTTGGCTCAACTCTGA

FIG. 7C

hP2X2d

CCACCATGGCCGCCGCCCAGCCCAAGTACCCCGCCGGGGCGACCGCCCGGCGCCTGGCCCGGG
GCTGCTGGTCCGCCCTCTGGGACTACGAGACGCCCAAGGTGATCGTGGTGAGGAACCGGCGCC
TGGGGGTCCTGTACCGCGCCGTGCAGCTGCTCATCCTGCTCTACTTCGTGTGGTACGTATTCA
TCGTGCARAAAAGCTACCAGGAGAGCGAGACGGGCCCCGAGAGCTCCATCATCACCAAGGTCA
AGGGGATCACCACGTCCGAGCACAAAGTGTGGGACGTGGAGGAGTACGTGAAGCCCCCCGAGG
GGGGCAGCGTGTTCAGCATCATCACCAGGGTCGAGGCCACCCACTCCCAGACCCAGGGAACCT
GCCCCGAGAGCATAAGGGTCCACAACGCCACCTGCCTCTCCGACGCCGACTGCGTGGCTGGGG
AGCTGGACATGCTGGGAAACGGCCTGAGGACCGGGCGCTGTGTGCCCTATTACCAGGGGCCCT
CCAAGACCTGCGAGGTGTTCGGCTGGTGCCCGGTGGAAGATGGGGCCTCTGTCAGCCAATTTC
TGGGTACGATGGCCCCAAATTTCACCATCCTCATCAAGAACAGCATCCACTACCCCAAATTCC
ACTTCTCCAAGGGCAACATCGCCGACCGCACAGACGGGTACCTGAAGCGCTGCACGTTCCACG
AGGCCTCCGACCTCTACTGCCCCATCTTCAAGCTGGGCTTTATCGTGGAGAAGGCTGGGGAGA
GCTTCACAGAGCTCGCACACAAGGGTGGTGTCATCGGGGTCATTATCAACTGGGACTGTGACC
TGGACCTGCCTGCATCGGAGTGCAACCCCAAGTACTCCTTCCGGAGGCTTGACCCCAAGCACG
TGCCTGCCTCGTCAGGCTACAACTTCAGGTTTGCCAAATACTACAAGATCAATGGCACCACCA
CCCGCACGCTCATCAAGGCCTACGGGATCCGCATTGACGTCATTGTGCATGGACAGGCCGGGA
AGTTCAGCCTGATTCCCACCATTATTAATCTGGCCACAGCTCTGACTTCCGTCGGGGTGGTAA
GGAACCCTCTCTGGGGTCCCAGCGGGTGCGGGGGGTCCACCAGGCCCTTACACACCGGTCTCT
GCTGGCCCCAGGGCTCCTTCCTGTGCGACTGGATCTTGCTAACATTCATGAACAAAAACAAGG
TCTACAGCCATAAGAAATTTGACAAGGTGTGTACGCCGAGCCACCCCTCAGGTAGCTGGCCTG
TGACCCTTGCCCGTGTATTGGGCCAGGCCCCTCCCGAACCCGGCCACCGCTCCGAGGACCAGC
ACCCCAGCCCTCCATCAGGCCAGGAGGGCCAACAAGGGGCAGAGTGTGGCCCAGCCTTCCCGC
CCCTGCGGCCTTGCCCCATCTCTGCCCCTTCTGAGCAGATGGTGGACACTCCTGCCTCCGAGC
CTGCCCAAGCCTCCACACCCACAGACCCCAAAGGTTTGGCTCAACTCTGA

FIG. 7D

hP2X2 polypeptide

MAAAQPKYPAGATARRLARGCWSALWDYETPKVIVVRNRRLGVLYRAVQLLILLYFVWYVFIV
QKSYQESETGPESSIITKVKGITTSEHKVWDVEEYVKPPEGGSVFSIITRVEATHSQTQGTCP
ESIRVHNATCLSDADCVAGELDMLGNGLRTGRCVPYYQGPSKTCEVFGWCPVEDGASVSQFLG
TMAPNFTILIKNSIHYPKFHFSKGNIADRTDGYLKRCTFHEASDLYCPIFKLGFIVEKAGESF
TELAHKGGVIGVIINWDCDLDLPASECNPKYSFRRLDPKHVPASSGYNFRFAKYYKINGTTTR
TLIKAYGIRIDVIVHGQAGKFSLIPTIINLATALTSVGVGSFLCDWILLTFMNKNKVYSHKKF
DKVCTPSHPSGSWPVTLARVLGQAPPEPGHRSEDQHPSPPSGQEGQQGAECGPAFPPLRPCPI
SAPSEQMVDTPASEPAQASTPTDPKGLAQL

FIG. 8A

hP2X2b

MAAAQPKYPAGATARRLARGCWSALWDYETPKVIVVRNRRLGVLYRAVQLLILLYFVWYVFIV
QKSYQESETGPESSIITKVKGITTSEHKVWDVEEYVKPPEGGSVFSIITRVEATHSQTQGTCP
ESIRVHNATCLSDADCVAGELDMLGNGLRTGRCVPYYQGPSKTCEVFGWCPVEDGASVSQFLG
TMAPNFTILIKNSIHYPKFHFSKGNIADRTDGYLKRCTFHEASDLYCPIFKLGFIVEKAGESF
TELAHKGGVIGVIINWDCDLDLPASECNPKYSFRRLDPKHVPASSGYNFRFAKYYKINGTTTR
TLIKAYGIRIDVIVHGQAGKFSLIPTIINLATALTSVGVGSFLCDWILLTFMNKNKVYSHKKF
DKMVDTPASEPAQASTPTDPKGLAQL

FIG. 8B

hP2X2c

MAAAQPKYPAGATARRLARGCWSALWDYETPKVIVVRNRRLGVLYRAVQLLILLYFVWYVFIV
QKSYQESETGPESSIITKVKGITTSEHKVWDVEEYVKPPESIRVHNATCLSDADCVAGELDML
GNGLRTGRCVPYYQGPSKTCEVFGWCPVEDGASVSQFLGTMAPNFTILIKNSIHYPKFHFSKG
NIADRTDGYLKRCTFHEASDLYCPIFKLGFIVEKAGESFTELAHKGGVIGVIINWDCDLDLPA
SECNPKYSFRRLDPKHVPASSGYNFRFAKYYKINGTTTRTLIKAYGIRIDVIVHGQAGKFSLI
PTIINLATALTSVGVGSFLCDWILLTFMNKNKVYSHKKFDKVCTPSHPSGSWPVTLARVLGQA
PPEPGHRSEDQHPSPPSGQEGQQGAECGPAFPPLRPCPISAPSEQMVDTPASEPAQASTPTDP
KGLAQL

FIG. 8C

hP2X2d

MAAAQPKYPAGATARRLARGCWSALWDYETPKVIVVRNRRLGVLYRAVQLLILLYFVWYVFIV
QKSYQESETGPESSIITKVKGITTSEHKVWDVEEYVKPPEGGSVFSIITRVEATHSQTQGTCP
ESIRVHNATCLSDADCVAGELDMLGNGLRTGRCVPYYQGPSKTCEVFGWCPVEDGASVSQFLG
TMAPNFTILIKNSIHYPKFHFSKGNIADRTDGYLKRCTFHEASDLYCPIFKLGFIVEKAGESF
TELAHKGGVIGVIINWDCDLDLPASECNPKYSFRRLDPKHVPASSGYNFRFAKYYKINGTTTR
TLIKAYGIRIDVIVHGQAGKFSLIPTIINLATALTSVGVVRNPLWGPSGCGGSTRPLHTGLCW
PQGSFLCDWILLTFMNKNKVYSHKKFDKVCTPSHPSGSWPVTLARVLGQAPPEPGHRSEDQHP
SPPSGQEGQQGAECGPAFPPLRPCPISAPSEQMVDTPASEPAQASTPTDPKGLAQL

FIG. 8D

```
                          10                  20                  30                  40                  50
hP2X2a pro  M A A A Q P K Y P A G A T A R R L A R G C W S A L W D Y E T P K V I V V R N R R L G V L Y R A V Q L
hP2X2b pro  M A A A Q P K Y P A G A T A R R L A R G C W S A L W D Y E T P K V I V V R N R R L G V L Y R A V Q L
hP2X2c pro  M A A A Q P K Y P A G A T A R R L A R G C W S A L W D Y E T P K V I V V R N R R L G V L Y R A V Q L
hP2X2d pro  M A A A Q P K Y P A G A T A R R L A R G C W S A L W D Y E T P K V I V V R N R R L G V L Y R A V Q L

                          60                  70                  80                  90                  100
hP2X2a pro  L I L L Y F V W Y V F I V Q K S Y Q E S E T G P E S S I I T K V K G I T T S E H K V W D V E E Y V K
hP2X2b pro  L I L L Y F V W Y V F I V Q K S Y Q E S E T G P E S S I I T K V K G I T T S E H K V W D V E E Y V K
hP2X2c pro  L I L L Y F V W Y V F I V Q K S Y Q E S E T G P E S S I I T K V K G I T T S E H K V W D V E E Y V K
hP2X2d pro  L I L L Y F V W Y V F I V Q K S Y Q E S E T G P E S S I I T K V K G I T T S E H K V W D V E E Y V K

                          110                 120                 130                 140                 150
hP2X2a pro  P P E G G S V F S I I T R V E A T H S Q T Q G T C P E S I R V H N A T C L S D A D C V A G E L D M L
hP2X2b pro  P P E G G S V F S I I T R V E A T H S Q T Q G T C P E S I R V H N A T C L S D A D C V A G E L D M L
hP2X2c pro  P P E - - - - - - - - - - - - - - - - - - - - S I R V H N A T C L S D A D C V A G E L D M L
hP2X2d pro  P P E G G S V F S I I T R V E A T H S Q T Q G T C P E S I R V H N A T C L S D A D C V A G E L D M L

                          160                 170                 180                 190                 200
hP2X2a pro  G N G L R T G R C V P Y Y Q G P S K T C E V F G W C P V E D G A S V S Q F L G T M A P N F T I L I K
hP2X2b pro  G N G L R T G R C V P Y Y Q G P S K T C E V F G W C P V E D G A S V S Q F L G T M A P N F T I L I K
hP2X2c pro  G N G L R T G R C V P Y Y Q G P S K T C E V F G W C P V E D G A S V S Q F L G T M A P N F T I L I K
hP2X2d pro  G N G L R T G R C V P Y Y Q G P S K T C E V F G W C P V E D G A S V S Q F L G T M A P N F T I L I K

                          210                 220                 230                 240                 250
hP2X2a pro  N S I H Y P K F H F S K G N I A D R T D G Y L K R C T F H E A S D L Y C P I F K L G F I V E K A G E
hP2X2b pro  N S I H Y P K F H F S K G N I A D R T D G Y L K R C T F H E A S D L Y C P I F K L G F I V E K A G E
hP2X2c pro  N S I H Y P K F H F S K G N I A D R T D G Y L K R C T F H E A S D L Y C P I F K L G F I V E K A G E
hP2X2d pro  N S I H Y P K F H F S K G N I A D R T D G Y L K R C T F H E A S D L Y C P I F K L G F I V E K A G E

                          260                 270                 280                 290                 300
hP2X2a pro  S F T E L A H K G G V I G V I I N W D C D L D L P A S E C N P K Y S F R R L D P K H V P A S S G Y N
hP2X2b pro  S F T E L A H K G G V I G V I I N W D C D L D L P A S E C N P K Y S F R R L D P K H V P A S S G Y N
hP2X2c pro  S F T E L A H K G G V I G V I I N W D C D L D L P A S E C N P K Y S F R R L D P K H V P A S S G Y N
hP2X2d pro  S F T E L A H K G G V I G V I I N W D C D L D L P A S E C N P K Y S F R R L D P K H V P A S S G Y N
```

FIG.9A

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                          310                 320                 330                 340                 350
hP2X2a pro  F R F A K Y Y K I N G T T T R T L I K A Y G I R I D V I V H G Q A G K F S L I P T I I N L A T A L T
hP2X2b pro  F R F A K Y Y K I N G T T T R T L I K A Y G I R I D V I V H G Q A G K F S L I P T I I N L A T A L T
hP2X2c pro  F R F A K Y Y K I N G T T T R T L I K A Y G I R I D V I V H G Q A G K F S L I P T I I N L A T A L T
hP2X2d pro  F R F A K Y Y K I N G T T T R T L I K A Y G I R I D V I V H G Q A G K F S L I P T I I N L A T A L T

                          360                 370                 380                 390                 400
hP2X2a pro  S V G V G - - - - - - - - - - - - - - - - - - - - - - - - S F L C D W I L L T F M N K N K V Y S
hP2X2b pro  S V G V G - - - - - - - - - - - - - - - - - - - - - - - - S F L C D W I L L T F M N K N K V Y S
hP2X2c pro  S V G V G - - - - - - - - - - - - - - - - - - - - - - - - S F L C D W I L L T F M N K N K V Y S
hP2X2d pro  S V G V G R N P L W G P S G C G G S T R P L H T G L C W P Q G S F L C D W I L L T F M N K N K V Y S

                          410                 420                 430                 440                 450
hP2X2a pro  H K K F D K V C T P S H P S G S W P V T L A R V L G Q A P P E P G H R S E D Q H P S P P S G Q E G Q
hP2X2b pro  H K K F D - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
hP2X2c pro  H K K F D K V C T P S H P S G S W P V T L A R V L G Q A P P E P G H R S E D Q H P S P P S G Q E G Q
hP2X2d pro  H K K F D K V C T P S H P S G S W P V T L A R V L G Q A P P E P G H R S E D Q H P S P P S G Q E G Q

                          460                 470                 480                 490
hP2X2a pro  Q G A E C G P A F P P L R P C P I S A P S E Q M V D T P A S E P A Q A S T P T D P K G L A Q L
hP2X2b pro  - - - - - - - - - - - - - - - - - - - - - - - K M V D T P A S E P A Q A S T P T D P K G L A Q L
hP2X2c pro  Q G A E C G P A F P P L R P C P I S A P S E Q M V D T P A S E P A Q A S T P T D P K G L A Q L
hP2X2d pro  Q G A E C G P A F P P L R P C P I S A P S E Q M V D T P A S E P A Q A S T P T D P K G L A Q L
```

FIG.9B

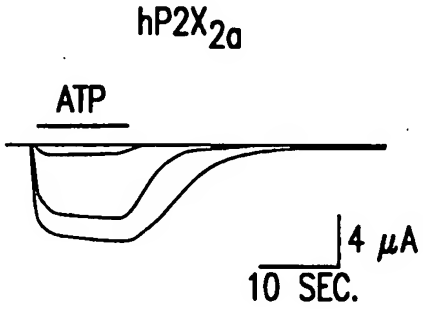


FIG. 10A

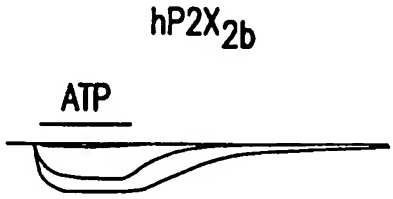


FIG. 10B

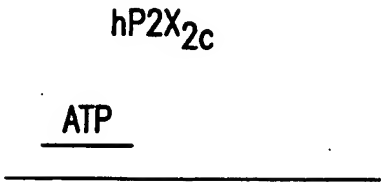


FIG. 10C

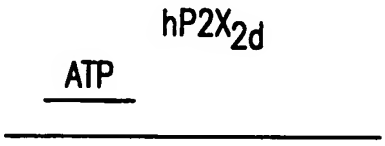


FIG. 10D

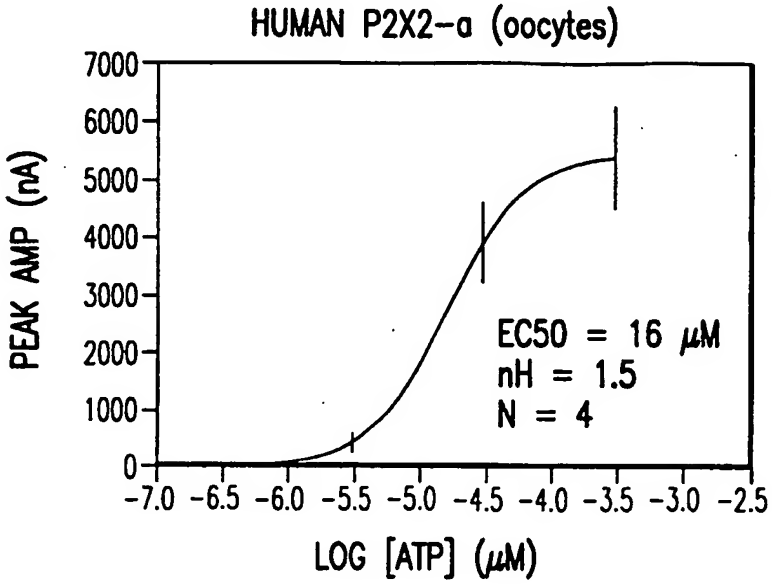


FIG. 10E

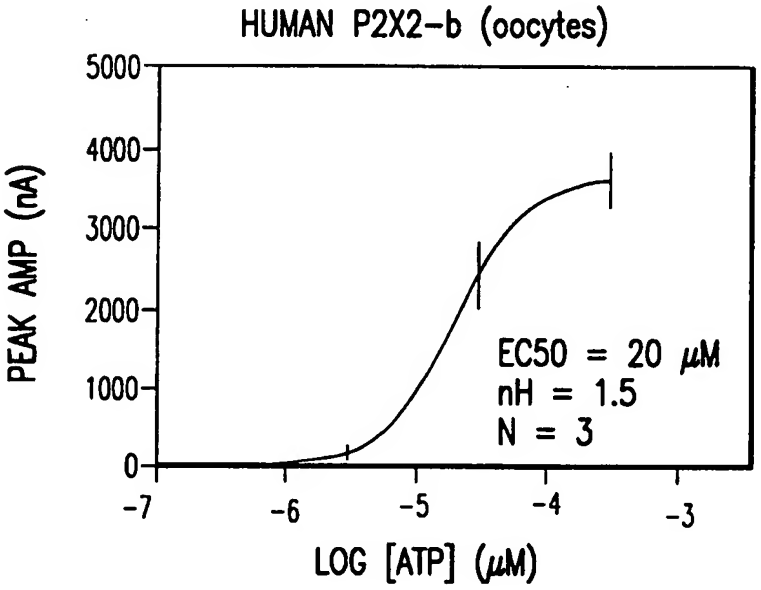


FIG. 10F